IMPROVED HATCHERY AND NURSERY REARING TECHNIQUES FOR *PECTEN FUMATUS* REEVE

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Fortnightly sampling of a population of the hermaphroditic scallop *Pecten fumatus* in Jervis Bay was initiated in July 1991. Results over the first 18 months showed that wild stocks constitute a poor and unpredictable source of ripe ready-to-spawn broodstock for hatchery use. This prompted development of hatchery conditioning protocols. The most rapid development of gonads occurred when broodstock were held at 15°C and fed to satiation ($6x10^9$ cells $scallop^{-1}$ day^{-1} on a diet of approximately equal amounts of at least 3 of 4 microalgae, namely: *Chaetoceros calcitrans, Pavlova lutheri,* Tahitian *Isochrysis* and *Chroomonas salina*). Intragonadal injection of serotonin at 0.05ml of a $0.5x10^{-4}$N solution per scallop reliably induced sperm release within 5–25min over a broad (12–24°C) temperature range. Survival from fertilisation to D-veliger stage was substantially improved by incubating eggs in suspension at up to 100 ml^{-1} in aerated cylindro-conical vessels. Survival to metamorphosis on Day 16 ranged from 5–20%. Rates up to 70% were achieved with experimental scale cylindro-conical rearers when seawater was prefiltered to 1m or when antibiotics were used. Post-settlement retention rates of 10-50% were achieved by transferring pediveligers onto cylindrical downweller screens fitted with 160µm polyester mesh. Growth of 5–10mm juvenile scallops maintained in an upweller nursery unit located at a site at the entrance to Port Stephens was found to increase with increasing seawater flow rates up to 40ml g^{-1} biomass min^{-1} and to be suppressed when the surface area of scallops approached 100% that of the screens on which they were stocked. Mean growth rates of 2.8 mm $week^{-1}$ were exhibited over the size range 5–25mm when maintained at low density in screens or lantern cages suspended from a long line at 20–24°C. Small spat in the hatchery grew faster with increasing temperature in the range 12–27°C but ceased growing at 1.5–3mm.

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The NSW scallop fishery is spasmodic and confined to Jervis and Twofold Bays (Fig.1). Peak annual catches of 1000–3000 tonnes live weight occur only once in 10 years with insignificant catches in intervening years (Fuentes et al., 1992). If higher and more consistent scallop yields are to be achieved in NSW, the central problem of low and variable annual recruitment of juveniles, must be addressed. Wild caught *Pecten fumatus* spat in Jervis Bay (and probably elsewhere in southern NSW) is likely to be low and unreliable in most years (Fuentes et al.,1992).

The importance of reliable, low-cost hatchery and nursery rearing techniques for *P. fumatus* and a successful pilot hatchery trial in May 1989 prompted a 3 year, Fishing Industry Research and Development Corporation (FRDC) funded, research project at the Brackish Water Fish Culture Research Station (BWFCRS) from July 1991.

The 1989 pilot study gave encouraging results using wild scallops from Jervis Bay as spawning stock and conventional hatchery rearing techniques and equipment (Frankish et al., 1991). Approximately 6 million settled spat were being produced at estimated survival rates from spawning to D-veliger (1st feeding) stage of c.60% and from D-veliger to post-settlement, of c.70%. Several hundred thousand settled spat were retained and onreared to 10–20mm shell height at a similar rate of survival (Frankish et al.,1990). These results contrasted with those previously attained by Tasmanian oyster hatcheries using comparable techniques and equipment. In attempting to meet Tasmanian government contracts for the supply of 4.2 million *P. fumatus* juveniles in the range 10–20mm, the hatcheries were only able to supply 100000 and 280000 in 1987 and 1988 respectively. Up to this time, the largest spawning of *P. fumatus* had produced 125 million eggs but no hatchery had produced more than 500000 settled spat from one batch of larvae (Cropp & Frankish,1989).

From the outset of this project, in 1991, it was considered that previous high variability in hatchery success with *P. fumatus* could have

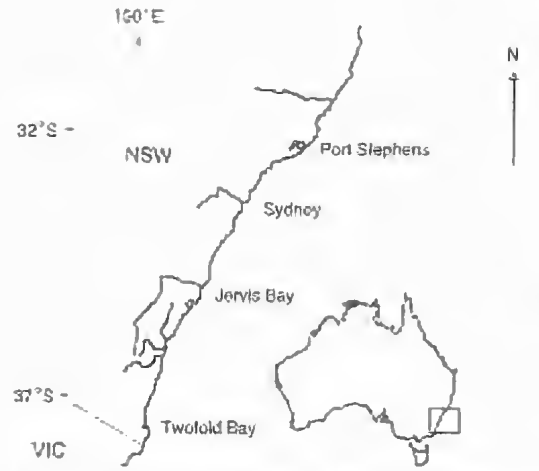

FIG. 1. Central and southern New South Wales.

arisen through one or a combination of several factors. These included: variability in the quality of eggs sourced from wild spawners; subtle, but critical differences in equipment and techniques employed, especially in relation to settlement and metamorphosis of pediveligers; disease(s) and larval nutrition factors.

MATERIALS AND METHODS

Evaluation of Wild Stocks of *P. fumatus* as a Source of Ready to Spawn Broodstock

Fortnightly sampling of a Jervis Bay population of *P. fumatus* was initiated in July 1991. On each occasion 120–150 scallops in the range 55–90 mm shell length were collected by a professional diver. All collections made between 7–8am were road freighted (insulated from an underlying layer of ice which maintained them at 10–15°C) to BWFCRS within 8–10hrs. They were immediately stocked into a lantern cage suspended in a 1000l holding tank at ambient temperature (16–22°C). The following morning 40 randomly selected scallops was measured, subjected to a macro-visual staging of gonad condition (Fuentes et al.,1992) and then dissected to determine gonad somatic index (GSI)[1].

$$GSI = \frac{Weight\ of\ gonad}{Total\ shell\ free\ drained\ weight} x100$$

Of the remaining 80–110 scallops, the 10 individuals exhibiting highest apparent gonad condition (degree of ripeness) were subjected to induction of spawning stimuli within 72h of capture. Induction of spawning stimuli comprised the exposure of scallops to 3 thermal cycles in which temperature was raised 3–8°C above ambient over periods of 45–60 minutes.

Release of sperm and eggs was recorded for this hermaphroditic species with fecundity being determined in the case of egg releases.

Gonad Conditioning Protocols for Captive Broodstock

Attempts to condition broodstock in the hatchery were conducted between July and September, 1991. Wild scallops with medium to high gonadal development attained a ripe, 'ready to spawn', condition in 4–6 weeks during July and August at 16–19°C and fed to satiation. Gonad condition regressed as temperatures rose above 20°C in September and October.

These results prompted construction of a controlled temperature broodstock conditioning facility at the BWFCRS to develop techniques that would enable controlled ripening, stockpiling and induced spawning of captive broodstock throughout the year. This facility, commissioned in April 1992, comprised 4 water baths held at 12.0±0.5; 15.0±0.5; 18.0±0.5 and 21.0±0.5°C respectively, each accommodating 36 x10 l plastic aerated aquaria to accommodate individual scallops. Experiments to identify appropriate microalgal diets and satiation feeding levels were initiated in June 1992. Subsequent trials to identify optimum combinations of holding temperature at 100, 50, 25 and 12% of satiation feeding levels were conducted in July 1992.

Spawning Induction and Incubation Protocols

A series of trials was conducted to determine whether intergonadal injection of the neurotransmitter serotonin is more effective than temperature shocks in triggering spawning of ripe *P. fumatus*. To establish optimum dosage rates, 0.05ml serotonin was injected at concentrations of 10^{-6} to 10^{-3} N. Time to spawning of sperm and eggs was recorded as was fecundity in the case of released eggs. Fertilised eggs were stocked into 11 cylindroconical vessels and incubated at densities of 1–100 eggs ml^{-1} to gauge the effect of stocking density on survival to the 'D' veliger stage 48h after fertilisation.

Larval Rearing Techniques

Standard hatchery techniques and equipment for Sydney rock oysters (*Saccostrea commercialis*) larvae (Frankish et al.,1991) were used over the first 12 months. Consistently poor hatchery survival achieved by these means and lack of suitable facilities with which to conduct

replicated trials to evaluate alternative rearing techniques, prompted construction of a small scale bivalve larvae rearing system in August 1992. Ten standard 1000l flat-bottomed cylindrical oyster larval rearing vessels were utilised as controlled temperature baths, each accommodating 4x80l cylindroconical rearers. As with the 1000l vessels, the smaller units were made of rotationally moulded polyethylene.

Experimental treatments comprised four types of seawater preparation ie. filtration to 0.2μm absolute; filtration to 1.0μm nominal; filtration to 1.0μm nominal plus 10mg l^{-1} chloramphenicol and an unfiltered seawater control. These were combined factorially with two alternative diets, namely, a standard blend of 3 microalgal species (*C. calcitrans*, Tahitian *Isochrysis*, and *P. lutheri*) and the same blend of microalgae concentrated into a slurry by centrifugation and stored for 1–6 days prior to feeding. The chloramphenicol treatment was to evaluate the claim that a closely related European scallop *P. maximus*, cannot be hatchery reared with consistent success without use of such powerful broad spectrum antimicrobials (Samain et al.,1992) and hence whether microbial pathogens posed a significant constraint to hatchery success with this species.

Inclusion of chloramphenicol as an experimental treatment should not be construed as an endorsement of its use. To the contrary, identification of alternatives to use of broad spectrum antibiotics that will ensure consistently high survival of hatchery reared *P. fumatus* has become the most important single objective of this project.

Larval Settlement and Early Nursery Rearing Protocols

Post settlement recovery of *P. fumatus* pediveligers using conventional plastic mesh catch (culch) materials and by transferring pediveligers directly onto downweller screens fitted with 160μm polyester mesh were compared. Subsequent growth and survival was monitored for post-larvae retained on downweller screens in the hatchery and for those transferred to upwellers screens and lantern cages at Tomaree Head, adjacent to the mouth of Port Stephens (32° 44'S; 152° 11'E; Fig. 1).

A trial to determine optimum stocking and water flow rates for small juvenile *P. fumatus* reared on upweller screens at Tomaree Head was initiated on Christmas Eve 1992. A total of about 20000 spat averaging 5.6mm and 30mg were stocked at 8 different densities (5 replicates per density) using 40 miniaturised upweller units each consisting of vertically nested stacks of 8 interlocking screens.

The salinity tolerance of 1–2mm juvenile scallops was investigated in the laboratory as were interactive effects of salinity and temperature on growth and survival.

Specialist Handling Techniques for Early Juvenile and Adult Scallops

Mechanical methods such as seawater jets and scrapers used to dislodge small (10mm) bysally attached *P. fumatus* from culch materials and nursery screens were found to cause injury and subsequent high mortality. To address this problem, the effectiveness of a number of irritant chemicals and physiological stress factors to induce detachment of 2–4mm juveniles was evaluated in fully replicated trials.

A comparable series of trials was also conducted to test the anaesthetic properties of a range of chemical compounds on mature *P. fumatus* of 55–75mm shell height. The aim was to identify quick and simple techniques of reducing stress and subsequent inadvertent spawning caused by routine handling and assessment of gonad status of hatchery conditioned scallops.

RESULTS AND DISCUSSION

Wild Stocks of *P. fumatus* in NSW as a Source of Ripe Brood Stock

Fortnightly sampling of a Jervis Bay population of *P. fumatus* (July 1991–December 1992) revealed that this potential source of ripe 'ready to spawn' broodstock is nearly always unproductive and unpredictable. Even when breeding condition was highest, as indicated by peak in mean gonad-somatic index values of 18–21%, very few individual scallops, including those in a ripe condition (large, turgid, glossy and richly coloured gonads) responded positively and predicably to conventional spawning induction stimuli. Of 300 (10/collection) apparently ripe scallops subjected to spawning induction stimuli over the first 18 months of the project, less than 4% were successfully induced to spawn eggs. Moreover, seasonal peaks in breeding condition, were not consistent from year to year. For example, recorded mean GSI values were highest between December 1991 and March 1992 (Summer) but were continuously low during May-September 1992 (late Autumn-mid Spring). This pattern varied from that of chronically low mean GSI values recorded by Fuentes et al. (1992) through Summer and early Autumn of the two previous years and coin-

cided with unseasonally high winter sea temperatures and unseasonally low summer sea temperatures.

Hatchery Conditioning Protocols

Experiments to identify appropriate microalgal diets, feeding rates and temperatures for gonadal growth were undertaken in June 1992. Results of microalgal clearance rate experiments indicated that *Pavlova lutheri*, Tahitian *Isochrysis* aff. *galbana*, *Chroomonas salina* and *Chaetoceros gracilis* are ingested by adult scallops at similar rates at 14, 18 and 21°C but more slowly at 11°C. Satiation feeding rate using diets containing approximately equal numbers of cells of these 4 species, was estimated as about $6x10^9$ cells per day for broodstock of 55–75mm shell height. Cell densities of *Tetraselmis suecica*, another microalga commonly used to feed bivalves, declined over the first 8h of the experiment but then fluctuated, indicating resuspension of undigested cells from the faeces.

In a subsequent 6 week conditioning experiment, egg production rate associated with inadvertent spawning, along with gonad size and condition factors, were found to be highest when broodstock were held at 15°C, lowest for scallops maintained at 21°C and of intermediate values at 12°C and 18°C. Across all these temperatures, feeding of individual scallops with twice daily algal rations of $3x10^9$ cells (100% satiation) and $1.5x10^9$ cells (50% satiation) produced higher gonad ratings and egg production rates than rations of $0.75x10^9$ cells (25% satiation) or $0.375x10^9$ cells (12.5% satiation).

Frequent inadvertent spawnings triggered by handling emphasised the need for conditioning equipment and protocols that minimise handling and other disturbance factors. No inadvertent spawning however, occurred at feeding rates of 25 or 12.5% of satiation at 12°C. Use of low temperatures combined with reduced feeding rates might therefore enable stockpiling of broodstock at prime reproductive condition.

Opportunistic use was made of near ideal water temperatures (14–16°C) in August and early September 1992 to condition 100 broodstock. Scallops were held in lantern cages suspended in 20,000l tanks in the bivalve hatchery at BWFCRS and fed to satiation on a diet comprising equal amounts of the previously cited 4 microalgal species. To reduce the incidence of inadvertent spawning, rations of microalgae were drip fed into the tanks. The impact of water changes was kept to a minimum and handling of stock was totally avoided. No inadvertent spawnings were recorded over this period.

After 4 weeks of conditioning, 30 scallops were randomly selected and subjected to attempted thermal induction of spawning. Of these, 10 spawned as males and 12 as females, the latter yielding 25 million eggs and thence 13 million D-veliger larvae.

Spawning Induction, Fertilization and Incubation Protocols

Attempts to induce spawnings in ripe *P. fumatus* sampled fortnightly from Jervis Bay, were almost always unsuccessful. This result was originally ascribed, at least in part, to inadequacy of a standard thermal spawning induction stimulus (exposure of scallops to successive temperature rise cycles of 3–8°C above ambient) to trigger spawning. This misconception was corrected when development of effective gonad conditioning protocols yielded broodstock that spawned viable eggs rapidly in response to the same thermal induction techniques.

A dose of 0.05ml intragonadal injection of a $0.5x10^{-4}$N serotonin solution induced sperm release within 5-25 minutes over a broad range of temperature (14–22°C) in scallops with moderate to high gonad development. As with thermal shock technique however, induced spawning of eggs using serotonin was found to be effective only in suitably conditioned broodstock.

Injection of serotonin was nevertheless found to have a distinct advantage over the thermal induction of spawning. In being able to induce spawning of individual scallops held in isolation from one another. This in turn enables better control over the timing and extent (sperm to egg ratios) of fertilisation and the reduction of self fertilisation in hermaphoditic species such as *P. fumatus*. This attribute may prove particularly useful if applied to induced triploidy programs in the future. Use of serotonin induction of spawning has been extended to Sydney rock oysters at the BWFCRS in anticipation of this application.

Results of preliminary incubation trials conducted in November 1992 indicated that survival from fertilisation to D-veliger can be substantially improved by use of aerated cylindroconical vessels in which eggs are kept in suspension rather than allowing them to settle in a monolayer on the floor of conventional flat bottom oyster larvae rearing vessels. Suspended incubation also enables eggs to be stocked at high densities (up to 100 ml^{-1}) without apparent impairment to survival.

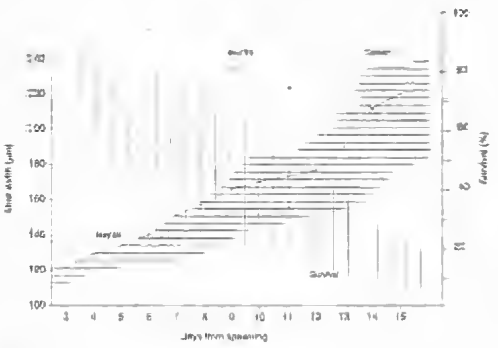

FIG.2. Summary of the growth and survival of hatchery reared scallops (*Pecten fumatus*) at BWFCRS, Salamander Bay. Dotted lines represent the results of the May 89 larval batch. Shaded areas indicate the range of results obtained from 6 larval batches conducted since July 91.

Larval Rearing Techniques

Larval rearing trials conducted over the first year of this project (Fig. 2) used standard 1000l flat-bottomed cylindrical tanks, used previously at the BWFCRS for the hatchery production of Sydney rock oysters (Frankish et al.,1991) and for the pilot hatchery production of *P. fumatus* in May 1989 (Frankish et al.,1990).

Survival to metamorphosis (Day 16–18) was 5–20% compared very poorly with the May '89 result but was in keeping with earlier results achieved by commercial hatcheries in Tasmania in 1987–1988 (Cropp & Frankish,1989). Larval growth rate varied and was not correlated with survival. Metamorphosis was attained at shell height of 225–240µm 14–20 days after spawning.

The first trial to systematically address the problem of low hatchery survival was undertaken in October 1992. Survival rates from D-veliger stage to the onset of metamorphosis (Fig. 3) varied with method of seawater preparation, being highest (70–80%) with chloramphenicol treated seawater and lowest (less than 10%) for 0.2µm filtered and unfiltered seawater. Larval survival rates were reduced but growth rates enhanced by algal concentrate diets. Subsequent patterns of survival through metamorphosis were however different with highest retention rates of 30–40% with 1µm filtered seawater and lowest rates of less than 1% with both unfiltered and 0.2µm absolute filtered seawater treatments.

These results highlighted the importance of continued research to identify seawater preparation, management protocols and feeding/stocking regimes that will enable consistent attainment of commercially acceptable hatchery survival rates. These are generally considered as nett yields of 0.2–1 of settled spat ml^{-1} of rearer volume (L. Goard, pers. comm.). The major challenge faced by continuing research is achievement of satisfactory yields of settled spat without the use of antibiotics.

Larval Settlement and Early Nursery Rearing Protocols

Of 7 larval rearing cycles completed during the first half of this project, 3 yielded significant numbers of spat. Approximately 15,000 spat were produced in November 1991; 30,000 in March 1992 and 200,000 in October/November 1992. Survival rates through settlement varied over the broad range 5–50%. Lowest survival was associated with the use of traditional plastic mesh culch materials deployed directly into larval rearing vessels. Much higher (10–50%) survival rates have however been consistently achieved by transferring pediveligers into downweller screens fitted with 160µm polyester mesh just prior to settlement.

Once settled onto downweller screens, subsequent mortality of spat was negligible. Growth of spat maintained in the hatchery is highly temperature dependent, increasing from c.15–150µm.day^{-1} with rising temperature over the range 12–27°C. Growth abruptly stalled as hatchery held spat attained a size of 1.5–3mm.

P. fumatus spat transferred to a longline unit at

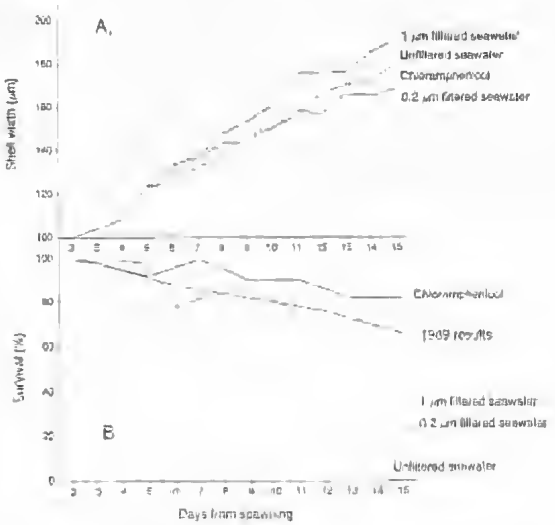

FIG.3. Effect of water treatment on growth and survival of *Pecten fumatus* larvae.

Tomaree Head, grew at mean rates of 2.8mm week^{-1} for sustained periods of up to 7 weeks at 20–23°C. This growth rate is higher than the previously highest summer rates of 1.7mm week^{-1} (Cropp,1985) for equivalent size *P. fumatus* glued to tapes suspended from midwater longlines in Tasmania.

A trial to determine optimum stocking rates of juvenile scallops reared in field upweller unit at Tomaree Head was initiated on Christmas Eve 1992. About 20,000 spat averaging 5.6mm and 30mg were stocked at 8 different densities (5 replicates per density) into 40 miniature upweller units, each of nested stacks of 8 interlocking screens.

Under prevailing conditions, including mean daily sea temperatures of 18–22°C and salinities of 34–35g.kg^{-1}, growth rate of spat of 6-10mm shell height and 30–150mg live-weight, was constrained by flow rates of below about 40ml g biomass^{-1} min^{-1}. For scallops in this size range, suppression of growth due to crowding coincided with a stocking density of about 0.3 g.cm^{-2} representing a surface area stocking rate approximating 100% of available screen area.

Salinity tolerance of 1–2mm juvenile *P. fumatus* was identified as a narrow range of 32–38mg ml^{-1} outside of which significant mortality occurs within 72h. These results were consistent with salinity tolerances reported for adult *P. fumatus* (Nell & Gibbs,1986).

Specialist Handling Techniques

Hypersaline baths (45 g.kg^{-1}) and exposure to air (emersion) for 2 hours were effective in inducing more than 95% of 1–3mm spat to detach from nursery screens. Hypersaline baths created by the addition of an artificial sea salt to seawater produced greater spat detachment after 2h than those created by equivalent additions of sodium chloride. The rate of detachment in hypersaline baths was unaffected by increasing temperature from 20–26°C, but was depressed at 11°C. Addition of magnesium chloride (27g.kg^{-1}) to seawater and reduction of seawater pH to 2 were also effective in increasing spat detachment rate, but not as effective as hypersaline baths or air exposure. With the exception of spat exposed to seawater containing 115mg.kg^{-1} available chlorine, no significant mortality and 95% reattachment occurred within 24h of all detachment methods tested.

Of 14 compounds tested, only chloral hydrate, $MgCl_2$ and $MgSO_4$ induced anaesthesia in adult scallops within 1h. $MgSO_4$ was excluded from further testing due to high postanaesthesia mortality. Doses of 4g.l^{-1} Chloral hydrate at 4g.l^{-1} (0.024M) or $MgCl_2$ at 30g.l^{-1} (0.31M) were most suitable on the basis of time to and recovery from anaesthesia. Neither anaesthetic caused mortality nor increased spawning activity. $MgCl_2$ reduced inadvertent spawning triggered by routine handling and maintenance activities. Time to anaesthesia for both agents was found to be affected ($P<0.05$) by water temperature.

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LITERATURE CITED

CROPP, D.A. 1985. Scallops thrive in TFDA culture program. Australian Fisheries 44(1): 16-18.

CROPP, D.A. & FRANKISH, K. 1989. Cost comparison of hatchery and natural scallop spat for the scallop *Pecten fumatus* Reeve. Pp. 196-225. In Dredge, M.L.C., Zacharine W.F. & Joll, L.M. (eds), 'Proceedings of the Australasian Scallop Workshop, Taroona, Tasmania, July 1988' (Tasmanian Government Printer: Hobart).

FRANKISH, K., GOARD, L. & O'CONNOR, W. 1990. Hatchery scallop culture success in NSW. Australasian Aquaculture Magazine 4(9): 10-11.

FRANKISH, K., GOARD, L. & O'CONNOR, W. 1991. 'The development of hatchery techniques for the production of Sydney rock Oysters (*Saccostrea commercialis*)'. Internal Report, NSW Fisheries, Brackish Water Fish Culture Research Station, 28p.

FUENTES, H.R., WILLIAMS, R.J., DIVER, L.P. & SMITH, A.K. 1992. Commercial molluscs of Jervis Bay. Pp. 000-000. In 'Jervis Bay marine ecological study. Final Report'. (NSW Fisheries, Fisheries Research Institute: Cronulla).

NELL, J.A. & GIBBS, P.J. 1986. Salinity tolerance and absorption of L- methionine by some Australian bivalve molluscs. Australian Journal of Marine and Freshwater Research 37: 721-727.

SAMAIN, J.F., SEGUINEAU, C., COCHARD, J.C., DELAUNAY, F., NICHOLAS, J.L., MARTY, Y., GALOIS, R., MATHIEU, M. & MOAL, J. 1992. What about growth variability for *Pecten maximus* production? Oceanis 18: 49-66.